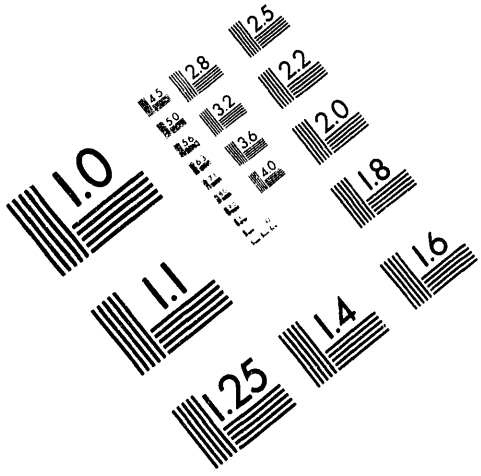

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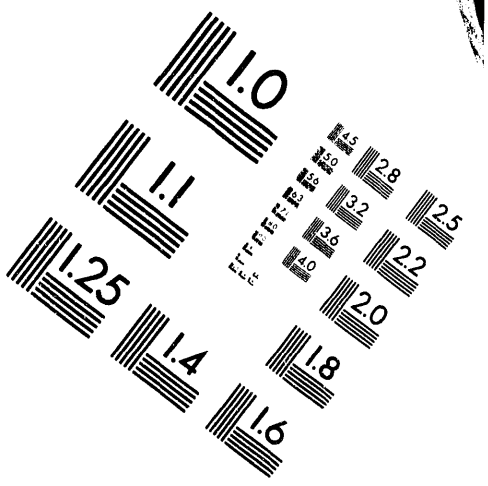

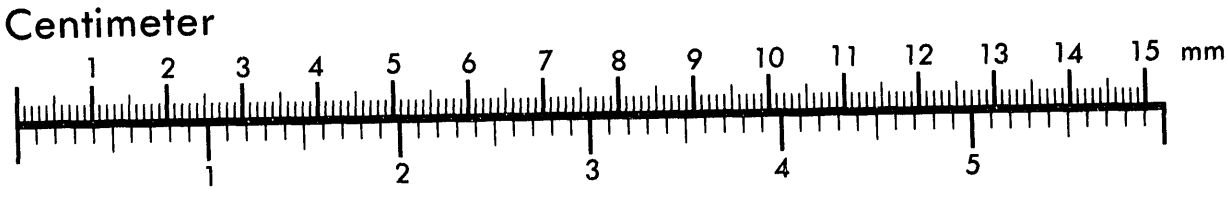

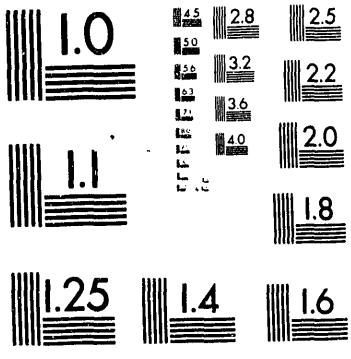

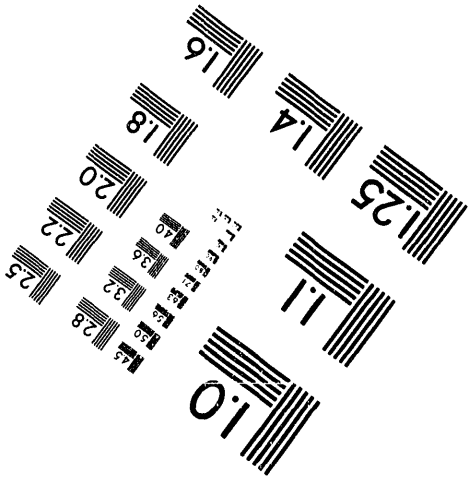

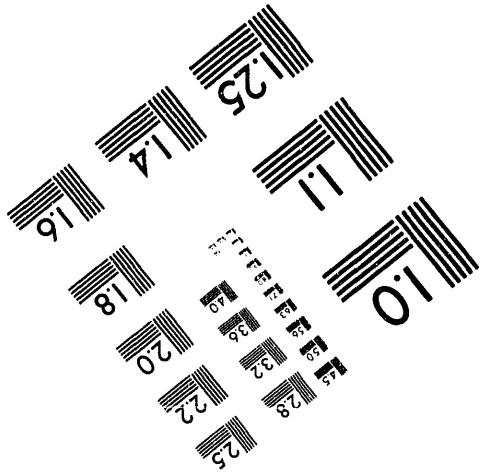

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ADVANCED BIOREACTORS FOR ENHANCED PRODUCTION OF CHEMICALS*

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ABSTRACT

A variety of advanced bioreactors are being developed to improve production of fuels, solvents, organic acids and other fermentation products. One key approach is immobilization of the biocatalyst leading to increased rates and yields. In addition, there are processes for simultaneous fermentation and separation to further increase production by the removal of an inhibitory product. For example, ethanol productivity in immobilized-cell fluidized-bed bioreactors (FBRs) can increase more than tenfold with 99% conversion and near stoichiometric yields. Two modified FBR configurations offer further improvements by removing the inhibitory product directly from the continuous fermentation. One involves the addition and removal of solid adsorbent particles to the FBR. This process was demonstrated with the production of lactic acid by immobilized *Lactobacillus*. The second uses an immiscible organic extractant in the FBR. This increased total butanol yields in the anaerobic acetone-butanol fermentation by *Clostridium acetobutylicum*.

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INTRODUCTION

Bioconversion processes utilize a biocatalyst (microorganism, enzyme, or other active fraction) to enhance the conversion of a feed material or substrate to a useful product in a controlled environment. It is particularly desirable for such a system to have high volumetric productivity with maximum concentration and yield of the product. Continuous operation with good process control is also desirable. At least two subcomponents need to be considered: the production of the bioreagent and the bioconversion reactor itself.

Most bioconversion processes utilize a soluble substrate in an aqueous solution and produce a product that is also soluble in the aqueous phase. However, the substrate can be a solid such as cellulose or starch, or even gases such as syngas or methane. Similarly the products can be solids, liquids, or gases. The reaction medium can be an aqueous solution, a moist gas, or even an organic liquid in contact with the biocatalytic component. An efficient biocatalyst system must be available in a bioreactor configuration that optimizes interphase contact, mass transport, and conversion kinetics.

Characteristics of an advanced bioreactor should include, if possible, high concentration of the biocatalyst, continuous operation, and excellent contact between the reacting components. The conventional bioreactor system today is a large stirred tank operating in the batch mode usually with microorganisms in suspension as the biocatalyst. After the tank is filled with the feed solution, the process typically requires a period of time when the biocatalyst is generated (microbial growth after an initial inoculum), followed by the actual bioconversion step after a sufficient concentration of the biocatalyst is present. The feed material or substrate is then removed and the product is separated from the fermentation broth. Thereafter, the system is cleaned out and the process begins again. The system may be sterilized between batches and the feed material may also be sterilized to prevent buildup of unwanted microbial contaminants, since the process may take many hours or even days.

Operation of such a system can be enhanced by utilizing continuous feed input and, thus, continuous product withdrawal as long as the feed rate is not high enough to completely wash the biocatalyst out of the system. This continuous stirred-tank reactor (CSTR) is also sometimes called a chemostat. A further enhancement is to remove the suspended biocatalyst from the reactor effluent and recycle it back to the reactor proper in order to significantly increase the biocatalyst concentration and, thus, increase the volumetric productivity of the system. This recycle can be achieved by centrifugation or with greater success by membrane filtration. Although there may be biological reasons that require batch operation (i.e., a sequence of required changes in the operating environment or a reaction that is intrinsically very slow), in most cases the CSTR with cell recycle should be considered the minimum for an advanced bioreactor system. The developmental challenge for this type of reactor system is the design of large tanks in which there is good interphase contact and mixing, the establishment of optimum operating conditions and controls, and the assurance of long-term aseptic operation.

An alternative to the conventional CSTR with cell recycle is the use of retained biocatalysts by immobilization onto integral parts of the reactor or by immobilization into or onto solid particles that will be kept in the bioreactor even at high flow rates. Two primary approaches can be used: 1) adsorption or attachment of the biocatalyst to external or internal surfaces of the solid phase; or 2) encapsulation of the biocatalyst within the particulate matrix or media.(1) This can result in a very high concentration of the biocatalyst that does not wash out of the bioreactor. Here the biocatalyst production step becomes a separate process for the production of large amount of biomass or enzymes. Although the retained-cell concept can be used in stirred-tanks, it is even more effective to utilize this concept in columnar bioreactors. Where long residence times are required, it is best to operate as a fixed-bed with larger particulates that are stationary in the reactor. For more rapid reaction, smaller particulates containing the biocatalysts can be suspended or fluidized in the column,

resulting in a fluidized-bed bioreactor. This latter type of reactor may well be the best solution if it provides sufficient residence time for conversion.

Membrane-type bioreactors can also be effective retained-cell systems. In this case, the biocatalyst is immobilized on one side of the membrane with contact with the substrate maintained across the membrane. Thus, the biocatalyst environment is isolated from the reaction environment.

Serious consideration is now being given the use of biocatalytic systems in or in contact with nonaqueous media. These primarily include organic solvents and supercritical liquids.(2) However, reactor concepts for these systems are only now being developed.

Many of the bioconversion steps (especially for the production of chemicals) are limited by conditions such as inhibitory product concentrations, deactivation of the biocatalyst and dilute aqueous streams, for example. Several proposed processes seek to alleviate these types of limitations by combining several processing steps together. Two good examples of combined processes are simultaneous saccharification and fermentation (SSF) and simultaneous fermentation and separation (SFS). There are several proposed concepts for SFS where the inhibitory product is removed from the ongoing bioconversion allowing higher conversions and rates. Some of these concepts are discussed in more detail below as a technology for the production of organic acids. Simultaneous saccharification and fermentation combines the enzymatic hydrolysis of cellulose with the bioconversion step. The enzyme cellulase is inhibited by its products glucose and cellobiose. SSF improves the rates of cellulase action by removing the sugars by fermentation into a less inhibitory product. SSF has been investigated for ethanol production.(3)

Many commodity chemicals can be produced by fermentation. Research at ORNL has emphasized those systems that operate continuously with high volumetric productivity are most promising. Columnar bioreactors with retained biocatalysts have been particularly attractive and three of these reactors are described and compared with other systems below.

Ethanol Production in a Fluidized-Bed Bioreactor

Immobilized *Zymomonas mobilis* were used in fluidized-bed bioreactors (FBR) for high productivity and conversion production of ethanol(4). The bacteria were immobilized within small uniform gel beads (~1 mm diam) at cell loadings of up to 50 g dry wt/L. Conversion and productivity were measured under a variety of conditions, feedstocks, flow rates, and column sizes (up to 8-ft tall). Volumetric productivities of 50 to 100 g EtOH/L-h have been achieved with residual glucose concentrations of less than 0.1%. The biocatalyst beads have been shown to remain active for over two months.

This technology has several advantages over conventional batch technology. Immobilization increases volumetric productivity by increasing cell density. The use of beads near 1 mm diam minimizes the effect of mass transfer resistances. Fluidization allows for good interphase mass transfer and the release of large volumes of coproduct CO_2. The columnar operation allows multistage operation and localizes the high inhibitory product concentrations to the top of the reactor. This would allow a much smaller reactor with smaller capital costs to be used for the same alcohol output.

Contamination is a serious problem in the long-term operation of many continuous bioreactors. Another advantage of this FBR was the operation without asepsis. Here, nonsterile operation was successful at pH 5 due to the high-flow rate and mixing removing the contaminants. A major advantage is the improved ethanol yield per gram dextrose of 0.49 g/g or >97% of the theoretical stoichiometric limit due to *Z. mobilis* compared to a yield of 0.45 to 0.47 g/g for yeast. Under current economic conditions the raw materials (i.e., dextrose from corn or other sources) are the largest single part of the cost; therefore, even a small but consistent increase in the yield can result in appreciable savings over the expected FBR operating lifetime of months.

Organic Acid Production and Removal

Many commercial organic acids can be produced by fermentation such as acetic, citric, lactic, and succinic acids.(5) All are produced in relatively dilute form due to their high level of inhibition of the microorganism. This inhibition is both due to the chemical itself and by the lowered pH from acid production. Improvements in rate have been observed using various means of cell retention including cell recycle, membranes, and immobilization.(6,7) These can lead to additional problems with mass transfer especially if oxygen is a required cosubstrate. Even with the increased rates, the final product concentration is comparable to batch reactions due to the inhibition. Conventionally, the fermentation broth is neutralized to control the pH. This yields the product in its salt form which requires additional processing to result in the desired acid.

Several processes have been proposed to remove the inhibitory product from the ongoing fermentation.(8) Precipitation of the organic acid salt can be done directly in the fermentation. However, the mode of retention must be considered to avoid separation problems with the precipitate. A major disadvantage of the precipitation method is the production of gypsum as a byproduct. Extraction by solvents has been proposed, both direct removal of the acid and a reactive removal by forming an ester in the organic solvent phase. Configurations for extraction have included STRs and membrane reactors. Adsorption has been proposed in various forms to remove the acid from the broth. This has included direct addition into the batch STR (with problems of attrition and power);(9) passing a broth recycle stream through a side adsorbent bed,(10) and a direct addition and removal of the adsorbent to a fluidized-bed of immobilized biocatalysts.(11)

This biparticle fluidized-bed bioreactor has been tested for simultaneous fermentation and separation of lactic acid. The bioreactor is a fluidized bed of immobilized *Lactobacillus delbreuckii.* Another solid phase of denser sorbent particles (a polyvinyl pyridine resin) was added to this fluidized bed. These sorbent particles fell through the bed, absorbed the product and were removed. In test fermentations, the addition of the sorbent enhanced the fermentation and moderated the fall of the

pH. The biparticle fluidized-bed bioreactor utilizing immobilized microorganisms and adsorbent particles has been shown to enhance the production of lactic acid fourfold in this nonoptimized system.

Extractive Bioconversion of Butanol

Butanol is a commodity chemical feedstock and solvent that, early in this century, was primarily made by industrial fermentation.(12) Butanol is the primary product of the fermentation of sugars by various bacteria, in particular *Clostridium acetobutylicum*. This is a complex fermentation, with, first, an acidogenic phase producing butyric and acetic acids and, then, a solventogenic phase producing butanol, acetone, and ethanol. Both the products and the lowered pH can be inhibitory to the continued fermentation. This has limited final butanol concentrations to a maximum of 15 g/L in batch culture. The removal of the inhibitory product from the ongoing fermentation has been suggested by many researchers as a method to alleviate the product inhibition and improve the process.(13,14)

The key advantages suggested for extractive bioconversion are: higher feed concentrations leading to less process wastes and reduced product recovery costs compared to distillation. Possibilities for *in situ* product removal include pervaporation,(15) the use of hollow-fiber reactors,(16) and the use of solid adsorbents (17) as well as the use of an immiscible extractive solvent. Key issues are the extractant toxicity and capacity as well as the actual contacting scheme devised and its operability.(18) Many solvents have been tested for the acetone-butanol fermentation.(16,19,20) Oleyl alcohol has been commonly used based on its low toxicity, reasonable distribution coefficient and selectivity for butanol.

Most studies of extractive acetone-butanol fermentation have been performed in a batch reactor(19) with free cells. Wayman and Parekh(20) performed a sequential batch extractive fermentation with cell recycle. A fed-batch fermentation with a concentrated glucose feed

continuously extracted the butanol from a recycled side stream and achieved a high butanol productivity of 1 g $L^{-1}h^{-1}$.(21) A CSTR recycled the cells with a membrane filter and provided a cell-free broth to an extraction cascade.(22) The lack of direct contact of the cells with the organic allowed the use of a more toxic extractant. An immobilized-cell fluidized bed bioreactor with a cocurrent immiscible liquid extractant(23) demonstrated a significant 50% to 90% increase in butanol production rate and yield in a nonoptimized extractive FBR system compared to the nonextractive FBR. The extractant oleyl alcohol removed most of the butanol from the aqueous phase during a active fermentation in a fluidized bed with immobilized *C. acetobutylicum* for the acetone-butanol fermentation. Under continuous, steady-state operation, the butanol yield increased to 0.3 g/g with a productivity of 1.8 g $L^{-1}h^{-1}$ when butanol was removed in this manner.

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